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ABNORMAL EGG STALK MORPHOLOGY IS CORRELATED WITH CLUTCH ATTRITION IN LABORATORY-MAINTAINED LOBSTERS (*HOMARUS*)

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Abstract

Female lobsters attach fertilized eggs to ovigerous setae on the pleopods by a stalk. Often a large percentage of the clutch is lost during the 6–12 month brooding interval, especially in laboratory-maintained females. The factors responsible for egg loss during brooding are undefined. We have compared the morphological characteristics of egg stalks from wild and laboratory-spawned females and correlated these characteristics with egg retention. Our data show that the morphology of egg stalks varies among laboratory-maintained lobsters and that there is a strong positive correlation between abnormal stalk morphology and clutch attrition. We conclude that improper formation of the egg stalk is a major cause of egg loss in laboratory-maintained lobsters.

Introduction

During spawning, female lobsters (*Homarus*) position themselves on their backs and extrude eggs through the gonopores located at the base of the third walking legs (Herrick, 1909). The eggs pass posteriorly to the abdomen where they attach to the ovigerous setae of the pleopods or to other eggs by means of a stalk (funiculus). The mechanism of egg attachment is controversial and incompletely understood (Aiken *et al.* 1980; Fisher and Clark, 1983; Goudeau and LaChaise, 1983). In *Homarus,* the stalk is continuous with the outer coat surrounding the egg (Harper and Talbot, 1983). The eggs are brooded and develop on the pleopods for 6–12 months before hatching. In wild populations, *H. americanus* females may lose up to 36% of a clutch before hatching occurs (Perkins, 1971). Clutch attrition in laboratory-maintained females is often much greater than this; some females drop all of their eggs before hatching (Talbot *et al.,* 1984).

There are probably numerous factors that account for egg loss, although few have actually been investigated. Extrinsic factors include predation on egg masses by a nemertean (Aiken *et al.,* 1980) and removal and/or eating of eggs by the female (Knight, 1918). Epibiotic bacteria are present on the surface of the egg coat and stalk of both wild caught and laboratory-maintained *Homarus* (Harper and Talbot, 1984). There is not a clear correlation between density of epibionts and egg loss from clutches, although excessively high bacterial loads may affect retention and/or embryonic development. Faulty attachment of the eggs is a factor which could result in clutch attrition, as the quality of the stalk would seem critical in proper retention over the long brooding period. Our purpose in this study was to examine the morphology of egg stalks from wild-spawned and laboratory-spawned females and to correlate structural characteristics of the stalk with egg retention. We show that stalk morphology varies among females and that females having egg stalks morphologically different from wild spawned females do not retain their clutches well.

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MATERIALS AND METHODS

Animals

Egg samples from 74 berried female lobsters (*Homarus americanus* and *H. gammarus*) were obtained from sources reported previously (Harper and Talbot, 1984). The females studied were of the following 5 types: (1) *H. americanus,* born in the wild and spawned in the wild (n = 9), (2) *H. gammarus,* born in the wild and spawned in the laboratory (n = 16), (3) *H. americanus,* born in the wild and spawned in the laboratory (n = 24), (4) *H. americanus,* born in the laboratory and spawned in the laboratory (n = 11), and (5) hybrids of *H. gammarus* ♀ x *H. americanus* ♂ and the reciprocal cross, which were both born and spawned in the laboratory (n = 14). Females in group 1 were considered controls, *i.e.,* they had spawned in the wild, and their eggs were presumed to be attached to the pleopods normally.

Processing of egg samples

Most samples were taken from the periphery of the clutch near the middle of the abdomen. Eggs were fixed in glutaraldehyde as described previously (Harper and Talbot, 1984). They were rinsed in cacodylate and either stored at 4°C in cacodylate buffer (pH 7.4, 0.1 *M*), or they were dehydrated in a graded acetone series and stored at 4°C in 100% acetone.

Relative abundance, density, and morphological characteristics of stalk material

Egg stalks were examined with a dissecting microscope to determine if morphological differences in stalk quality existed among females in the five groups. After preliminary examinations, several descriptive terms were found useful in evaluating these stalks. The relative amount of stalk material was characterized as: (1) abundant: eggs were interconnected by large amounts of stalk material, (2) moderate: a significant amount of stalk material was present, but less than in the abundant category, and (3) sparse: very little stalk material was present (see Figs. 1–3 for examples).

The density of the stalk material was characterized by its ability to transmit light. Samples were ranked as: (1) opaque, (2) translucent, or (3) transparent. These evaluations were made on flat regions of the stalk, usually close to its attachment to the egg.

The stalks were also evaluated for the following morphological characteristics: (1) twisted: a portion of most stalks was twisted, (2) flat: large, flat expanses of stalk material were frequently seen, (3) broad: the interface between the egg coat and stalk was large, (4) wispy: the stalk had a delicate, fragile appearance, and (5) thin: the stalk was unusually narrow in diameter.

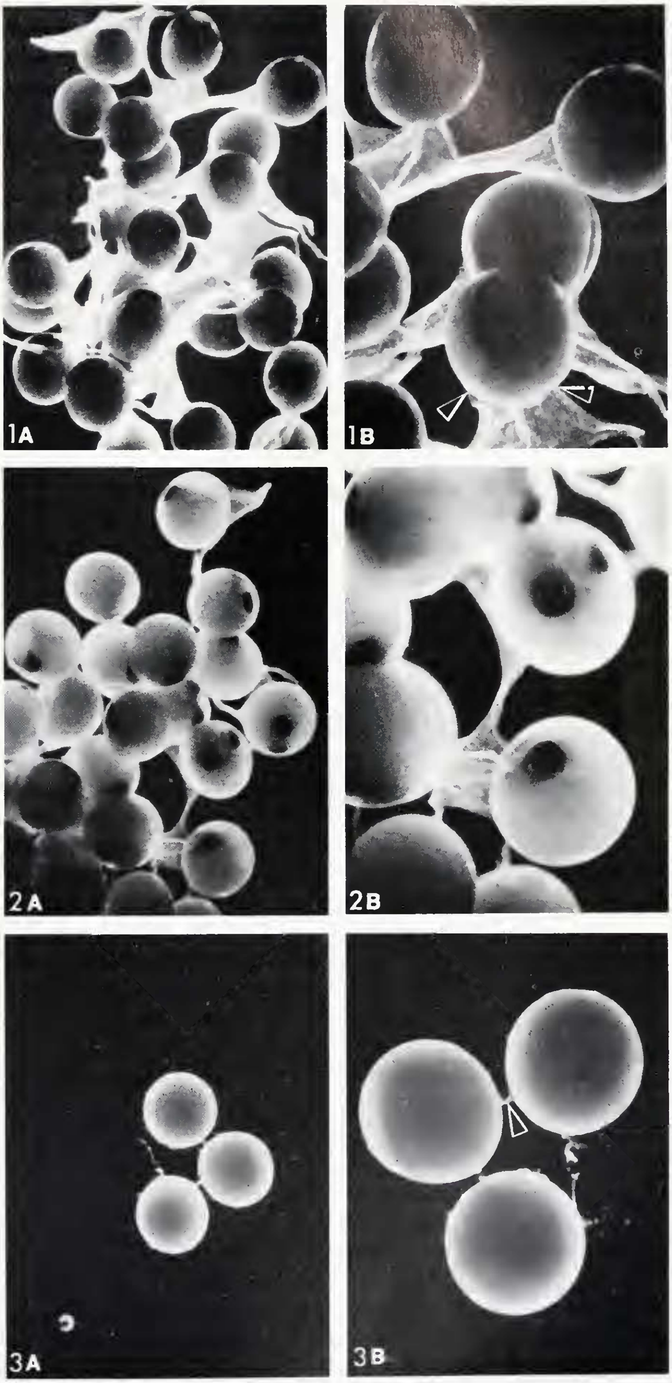
Egg retention

Records were kept at the laboratories of origin on egg retention for each female. These records were used to correlate egg retention with the observed morphological characteristics of the stalks for each female. All morphological data were collected before retention data were received making this aspect of the study blind. For some females, the number of eggs in clutches was estimated as described previously (Talbot *et al.,* 1984).

RESULTS

Relative abundance of stalk material

The amount of stalk material in each egg sample was rated as abundant, moderate, or sparse (Fig. 4). The wild born/wild spawned *H. americanus* and wild born/lab



FIGURES 1–3. Egg samples with stalk material evaluated as abundant (Fig. 1a, b), moderate (Fig. 2a, b), and sparse (Fig. 3a, b). The lower magnifications (Figs. 1a, 2a, 3a) illustrate differences in relative amount of stalk material. The higher magnifications (Figs. 1b, 2b) illustrate regions of stalks which were twisted, flat, and broad (attachment interface between arrows in Fig. 1b). The stalks in Figure 3b were evaluated as thin and wispy. Stalks in Figures 1b and 2b were evaluated as translucent.

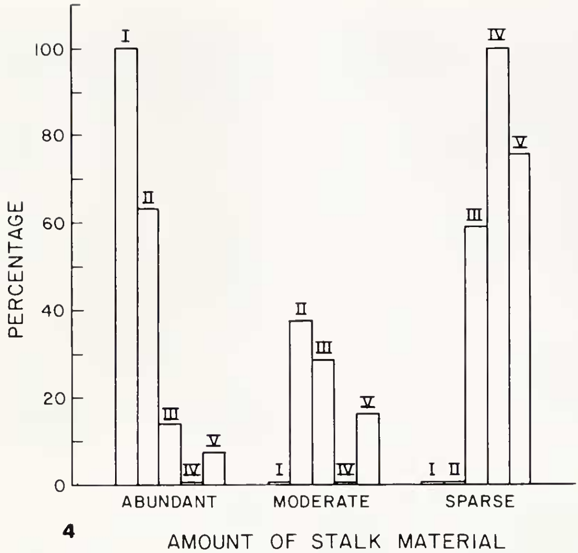


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FIGURE 4. The percentage of females in each of the 5 groups having stalks evaluated as abundant, moderate, or sparse. I = wild born/wild spawned *H. americanus;* II = wild born/lab spawned *H. gammarus;* III = wild born/lab spawned *H. americanus;* IV = lab born/lab spawned *H. americanus;* V = hybrids of *H. gammarus* × *H. americanus.*

spawned *H. gammarus* females all had abundant or moderate ratings. In contrast, most females in both groups of lab spawned *H. americanus* and the hybrid females had sparse amounts of stalk material.

Stalk density

The density of the stalk, as estimated by its opacity to light, is compared in Figure 5 for the 5 groups of females. Most wild born/wild spawned *H. americanus* and wild born/lab spawned *H. gammarus* had translucent stalks. However most stalk samples from females in the other 3 categories were rated transparent, suggesting they were thinner.

Morphological characteristics of the stalk material

The morphology of the stalks was evaluated for each egg sample using the descriptive terms twisted, flat, broad, thin, and wispy (Fig. 6). In almost all samples, some stalks were twisted. A high percentage of wild born/wild spawned *H. americanus* and wild born/lab spawned *H. gammarus* had stalks classified as broad and flat. In contrast, females in the other 3 groups most often had stalks which were thin and wispy.

Egg retention and quality of stalks

We have compared the relative abundance of the stalk material to egg retention times for each group of laboratory-maintained female (Table I). *H. americanus* females which were wild born/lab spawned showed variable results. Over 50% of these females had stalks evaluated as moderate or abundant. Egg loss did occur from these clutches,

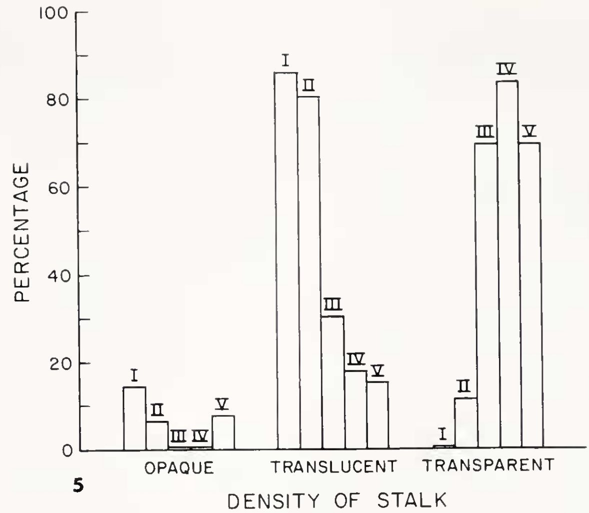

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FIGURE 5. The percentage of females in each of the 5 groups having stalks evaluated as opaque, translucent, or transparent. I = wild born/wild spawned *H. americanus;* II = wild born/lab spawned *H. gammarus;* III = wild born/lab spawned *H. americanus;* IV = lab born/lab spawned *H. americanus;* V = hybrids of *H. gammarus* × *H. americanus.*

and with the exception of one female (645;BML), loss was gradual over time. Eighty percent of the females carried at least a portion of their clutch through to hatching. Within the same group, 44% had stalks evaluated as sparse, and all except B-136 lost their eggs rapidly.

H. americanus females which were lab born/lab spawned all had sparse stalks (Table I), except for one female (R-130) which was evaluated as sparse/moderate.

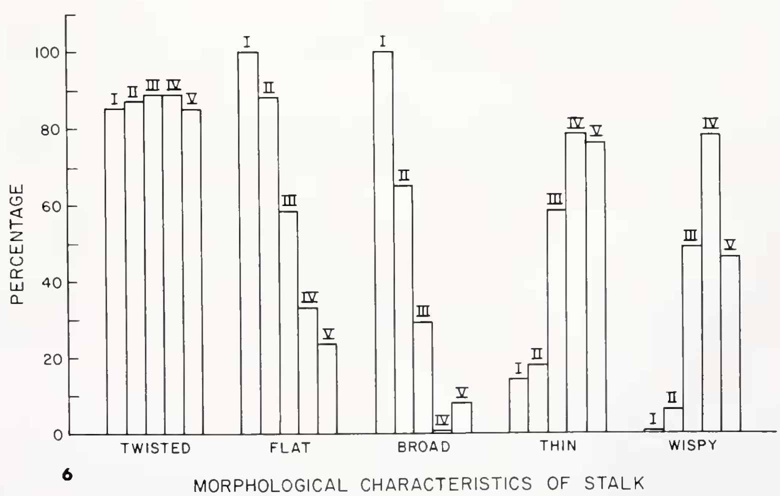

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FIGURE 6. The percentage of females in each of the 5 groups having stalks evaluated as twisted, flat, broad, thin, or wispy. I = wild born/wild spawned *H. americanus;* II = wild born/lab spawned *H. gammarus;* III = wild born/lab spawned *H. americanus;* IV = lab born/lab spawned *H. americanus;* V = hybrids of *H. gammarus* × *H. americanus.*

TABLE I

Amount of stalk material and egg retention

Stalk evaluation	% Attaching large clutches	% Gradual loss	% Rapid loss	% Hatching[1]
Wild Born/Lab Spawned *H. americanus* (n = 18)				
number abundant = 5 number moderate = 5	—	90%	10%	80%
number sparse = 8	25%	12.5%	87.5%	12.5%
Lab Born/Lab Spawned *H. americanus* (n = 6)				
number abundant or moderate = 0	—	—	—	—
number sparse = 6	16.7%	16.7%	83%	0%
Hybrids (n = 9)				
number abundant = 1	—	—	100% (eggs unfertilized)	0%
number sparse = 8	66.6% (n = 6)	0%	100%	0%
Wild Born/Lab Spawned *H. gammarus* (n = 13)				
number moderate or abundant = 13	100% (n = 7)	85%	15%	77%
number sparse = 0	—	—	—	—

[1] "Hatching" indicates that some eggs in the clutch were carried through to hatch.

All females, except R-130, attached 500 or fewer eggs, and these were lost rapidly. Female R-130 attached a large clutch (14,200) and lost all her eggs gradually before hatching.

Of the 9 hybrids, all except female 4011 had sparse stalks. Although most (67%) females attached a large clutch, eggs were lost rapidly and none were carried through to hatching. Female 4011 attached a small clutch of unfertilized eggs (she had not been mated) and lost these rapidly.

All *H. gammarus* females (n = 13) had moderate/abundant stalk evaluations. Initial clutch size data were available for 7 females, and all of these attached large clutches. Eleven of the 13 experienced gradual attrition; 1 of 13 lost her eggs rapidly and no data on attrition were available for the other female. Of the 13 females studied, 10 carried at least a portion of their clutch through to hatching.

Effect of fertilization on stalk morphology

Egg samples from 70 females were examined for evidence of fertilization. Eggs were considered fertilized if normal cleavage patterns or eye spot stages were observed. All wild born/wild spawned females had fertilized eggs and abundant stalks. For laboratory-maintained females, 49 of 63 females had fertilized eggs; 10 of 63 had eggs which could not be categorized unequivocally; and 4 of 63 females had eggs which were not fertilized. For the 4 unfertilized samples, stalks were categorized as abundant in 2 cases, moderate in one case, and undetermined in one case. Moreover, all hybrid females and all lab born/lab spawned females which had fertilized eggs produced stalks rated as "sparse." We conclude that production of "sparse" stalks was not caused by failure of the eggs to be fertilized.

Discussion

We have examined morphological characteristics of egg stalks from wild- and laboratory-maintained lobsters (*Homarus*) and correlated these characteristics with egg retention. Our data show that the morphology of the egg stalk varies among different groups of laboratory-maintained females. For control females (wild born/wild spawned *H. americanus*), stalks were most often evaluated as abundant, translucent, twisted, flat, and broad. Females having stalks of this type generally carry many eggs through to hatching, suggesting these are morphologically advantageous characteristics.

Laboratory-maintained females showed variations in the morphology of their egg stalks. All *H. gammarus* and about half the wild born/lab spawned *H. americanus* had stalks resembling those of the control group and with a few exceptions, such females carried some eggs through to hatching. This indicates that laboratory-maintained females producing stalks of the wild type are able to carry their clutches over the long brooding interval and supports the idea that stalk morphology affects egg retention. However, even in these clutches, attrition occurred. In some, but not all, instances, it was probably no greater than would be expected in a wild population (36%, Perkins, 1971). Thus, while good stalks are essential in clutch retention, even females with good stalks may experience considerable egg loss. The factors contributing to loss in such cases are probably subtle and should be subjected to further investigation.

The majority of laboratory-maintained females had stalks that were morphologically different than those of the controls. Females with stalks ranked as sparse included about half the wild born/lab spawned *H. americanus*, all the lab born/lab spawned *H. americanus*, and all the hybrids. These females often attached small clutches which they lost rapidly (both groups of *H. americanus*) or attached large clutches which they lost rapidly (hybrids). We conclude that much of the egg loss observed in laboratory-maintained females (Talbot *et al.*, 1984) can be attributed to the production of morphologically abnormal stalks.

Egg retention in captive, and perhaps in wild, females could be improved by more consistent production of wild-type egg stalks. The origin of the egg stalk has been the subject of controversy for years (see Fisher and Clark, 1983; Goudeau and La Chaise, 1983). In *Carcinus* (Goudeau and LaChaise, 1983) and in the lobster *Jasus* (Silberbauer, 1971) the egg stalk forms from an investment laid down while the egg is in the ovary. In *Homarus*, the egg stalk is formed from the chorion, a coat present around ovarian oocytes and comparable to the vitelline envelope of *Carcinus* (Talbot, 1983). The "sparse" stalks observed in this study may have resulted from incomplete or faulty synthesis of the chorion by some laboratory-maintained females. The chorion in *Homarus* is produced by follicle cells (Talbot, 1981), but the factors which regulate chorion synthesis are not known. Laboratory conditions in this study did not simulate wild conditions, and female reproductive physiology may be erratic in the higher ambient water temperatures found in some laboratories. Waddy and Aiken (1983) have shown that a winter water temperature of less than 10°C increases the percentage of females extruding eggs. It is also possible that environmental factors such as water temperature indirectly affect completion of chorion synthesis. This could be tested by maintaining laboratory females on a photoperiod and at a water temperature comparable to that of their native environment. Other factors, such as an incomplete hardening of the chorion after fertilization, may also influence proper formation and functioning of the egg stalk.

We conclude that the production of abnormal egg stalks is a major, but not the only, factor contributing to clutch attrition in laboratory-maintained lobsters and that the prevention of egg loss will be attained through a better understanding and control of chorion synthesis and formation of the egg stalk.

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